REMARKS/ARGUMENTS

Reconsideration of this application and entry of this Amendment, including the concurrently filed Terminal Disclaimer, are solicited. Claims 1-5 and 8 will be active in the application subsequent to entry of this Amendment.

Attached is a Terminal Disclaimer in respect of U.S. Patent 6,468,416 in order to resolve the obviousness-type double patenting rejection (paper no. 4).

The claims have been amended in order to more particularly point out and distinctly claim that which applicants regard as their invention. Claims 1 and 8 are amended to specify that the tetrazolium salt is WST-1 as described throughout applicants' specification and identified as a preferred tetrazolium at page 11, lines 13-20. In addition, components (a), (b) and (c) of claim 8 have been amended to specify immobilization responding to the rejection on page 2, third and fourth paragraphs, of the Official Action. A spelling error is apparent in claims 6 and 7 as pointed out on page 4 of the Official Action, and these claims have been withdrawn as being redundant considering the amendments made to claims 1 and 8.

This leaves for consideration the two prior art-based rejections of claims 4 and 5 as anticipated and claims 1-3 and 8 as being "obvious" over the indicated prior art documents. Applicants' comments are as follows:

US 5,639,672 (John F. Burd)

Burd reports a measurement method for fructosamine. More specifically, it is described that fructosamine is electrochemically measured by directly subjecting it to electrophoresis and enzymatic reaction with the aid of a mediator substance such as nitro tetrazolium blue salt (NBT).

a) Burd's method is used to measure only fructosamine, whereas the present invention can be used in quantifying dehydrogenase activities and various substrates.

b) Fructosamine produces eneaminol having a strong reducing power in a high alkaline pH region. Eneaminol directly and gradually reduces a nitro blue tetrazolium salt

(NBT) to produce formazan, which is colorimetrically detected. This method has been reported in many documents (for example, Clinica Chimica Acta. Vol. 127, p.87 (1982); and Clinical Chemistry, Vol. 33, p.2153 (1987)) published before Burd. This method is now generally used as a fructosamine measurement method in clinical tests.

c) Burd et al. as the inventors, know the reaction very well. In US 5,470,752 (page 3, lines 39 to 43 and page 5, lines 10 to 27 – copy attached) filed before the '672 patent, they describe a method of measuring fructosamine by producing formazan from a tetrazolium salt in an alkaline region without requiring an enzymatic reaction, similar to the method mentioned above.

d) The fructosamine measurement method can be carried out using a tetrazolium salt alone. In spite of this, if a measurement method employing three reaction substances (dehydrogenase. Coenzyme, and electron mediator) other than a tetrazolium salt is developed, it may not be used in most cases. This is because reagent cost increases and the measurement operation becomes more complicated. Therefore, a person skilled in the art with knowledge of Burd would not think of using a tetrazolium salt in combination with an electron mediator as disclosed and claimed in the present invention.

e) The tetrazolium salts disclosed in US 5,639,672 (Burd et al.) have been long investigated. The art considers that formazan is irreversibly produced through a reaction from a tetrazolium salt (Angew. Chem., Vol. 64. p.391 (1952); and Ber. dtsch. Chem. Ges., Vol. 77. p. 591 (1944)).

f) In the case of the irreversible reaction, the redox potential cannot be easily measured. Therefore, a special measurement technique as described in Chem. Reviews, Vol. 3, p.1 (1926) is required. Actually, the redox potential of a tetrazolium salt is investigated in Ber. dtsch. Chem. Ges., Vol. 77, p.591 (1944). In this case, since the redox potential is not measured directly by an electrode (no peak redox potential), the potential is measured by using an indicator. From this, it is found that a tetrazolium salt disclosed in US 5,639,672 (Burd et al.) cannot be measured electrochemically because of the absence of a redox peak potential.

g) When the present inventors also studied electrochemical measurement of a tetrazolium salt, NBT recited in claim 10 of US 5,639,672 (Burd et al.) was checked. As a result of this checking, no peak oxidation potential was observed in the range of -1000 mV to +1000 mV (vs. Ag/AgCl).

h) US 5,639,672 (Burd et al.) does not disclose an electrochemical measurement method for formazan produced from a tetrazolium salt. An electrochemical measurement method for fructosamine by applying a potential of +1040 mV (vs. Ag/AgCl) directly to fructosamine is only described in the Example. However, actually, no mention is made of formazan measurement.

i) WST-1 used in the present invention is a water-soluble tetrazolium salt recently (1993) developed. Therefore, whether WST-1 is electrochemically measured or not has not been sufficiently studied. WST-1 is not included in the tetrazolium salts mentioned in US 5,639,672 (Burd et al.).

j) When measurement is performed by applying a potential of +1040 mV (vs. Ag/AgCl) to a working electrode (glassy carbon), the measurement is affected by a solvent, water, (since the potential is close to the limit of the potential window region). Therefore, generally, it is difficult to measure at this potential. Furthermore, when a specimen such as blood is measured at this potential, interference substances contained in the blood may be greatly affected because of high potential application.

k) The formazan produced from a tetrazolium salt described in US 5,639,672 (Burd et al.) differs in substance from that produced from WST-1 used in the present invention. Applicants confirmed that only formazan derived from WST-1 exhibits an intrinsic oxidation peak potential.

l) In the present invention, formazan produced from WST-1 was actually quantified electrochemically. As a result, it is confirmed that the formazan from WST-1 exhibits an intrinsic oxidation peak potential at +500 mV (Ag/AgCl) (Fig. 2). Based on this, the electrochemical quantification method was established. Further, an immobilization method (Fig. 1) was developed, a biosensor was constructed, and further

investigations were performed. The results are shown in Figs. 3 and 4.

m) In addition, WST-1 used in the present invention is water soluble. The produced formazan also exhibits a good solubility (see EP 0,908,453 and US 6,130,054). However, the formazan produced from a tetrazolium salt shown in US 5,639,672 (Burd et al.), particularly, NBT (recited in the claims), is rarely dissolved in water (EP 0,908,453). As a result, there is a high possibility that formazan precipitates. When formazan precipitates, the electrode response decreases. As a result, it becomes impossible to construct a biosensor.

n) As explained above, US 5,639,672 (Burd et al.) describes a method of measuring formazan produced from a tetrazolium salt and a biosensor, but its disclosure is insufficient. In addition, there is a significant problem in constructing a biosensor using formazan. The present invention uses WST-1, which is not described in US 5,639,672 (Burd et al.), to overcome problems in establishing an electrochemical quantification method by applying a low potential and constructing a biosensor. In these respects, the present invention is patentable.

EP 0,908,453 (Ishiyama et al)

Ishiyama is directed to improvement of a conventional tetrazolium salt producing hardly soluble formazan, which will precipitate on a measuring device. As a result, a tetrazolium salt (as to WST-1, see JP 7,070,092) forming a water soluble formazan was developed. Furthermore, using such a tetrazolium salt, a fundamental study on absorbance measurement by NADH was carried out.

a) As described in paragraphs e) and f) of US 5,639,672 (Burd et al), since formazan is generally produced from a tetrazolium salt through an irreversible reaction, it has been considered that formazan cannot be measured electrochemically. EP. 0,908,453 (Ishiyama et al.) is also silent about such an electrochemical measurement method.

b) Ishiyama et al. have published a paper regarding WST-1 in Analyst Vol. 120, p.113 (1995) (corresponding to WST-1:lb). This paper is concerned with a cyclic voltammetric response using an electrode (p116. Fig. 5) and states that no oxidation peak

potential is obtained due to the irreversible reaction.

c) Judging from this paper, Ishiyama et al. seem to think that formazan is irreversibly produced from a conventional tetrazolium salt and therefore electrochemical measurement of formazan cannot be attained. From this, it is recognized that Ishiyama et al would never think of electrochemical measurement and the construction of a biosensor.

d) The present inventors found the oxidation peak potential of WST-1 at +500 mV (vs. Ag/AgCl) (Fig. 2) by applying a potential higher than +400 mV (vs. Ag/AgCl) disclosed in the Ishiyama's paper. Based on this, applicants have successfully established an electrochemical measurement method and constructed a biosensor.

e) As explained above, US 5,639,672 (Burd et al.) describes an electrochemical measurement for formazan produced from a tetrazolium salt. However, a method of measuring formazan electrochemically was not investigated at all. Also, the disclosure seems to be insufficient with respect to potential application. Because of its irreversible reaction, it is considered impossible to electrochemically measure the formazan produced from a tetrazolium salt. Therefore, even though Ishiyama et al performed cyclic voltammetry, it is recognized that they do not intend to establish electrochemical measurement method and construct a biosensor, from the paper described in Analyst, Vol. 120, p. 113 (1995). For these reasons, the present invention is not obvious over even if EP. 0,908,453 (Ishiyama et al.) is combined with US 5,639,672 (Burd et al.). Hence applicants' claims are patentable.

Although not applied against any of the pending claims, the current Action cites a new document regarded by the examiner to be pertinent to applicants' disclosure. The following comments are provided.

US 6,130,054 (Iwata)

Iwata describes a method of manufacturing a test strip for measuring the activity of creatine kinase and like enzymes. The test strip is formed by immobilizing at least a dehydrogenase, diaphorase, NAD or NADP, and a water soluble tetrazolium salt (WST-1) thereon. It is also described that the quantification is performed by measuring light

reflection.

a) Iwata et al is concerned with a general measurement method for a tetrazolium salt by quantifying formazan produced through various reactions, based on reflection light. No mention is made of electrochemical measurement.

b) Iwata et al states that at least a dehydrogenase, diaphorase, NAD or NADP, and a water soluble tetrazolium salt (claim 1) are adsorbed onto a test strip. In the present invention, 1-methoxy PMS is used in place of diaphorase and immobilized on an electrode (p. 12, lines 15 to 18). In this respect, the present invention differs from Iwata et al.

c) In the present invention, 1-methoxy PMS is used as an electron mediator in place of diaphorase. This is because the reactivity of 1-methoxy PMS is better than that of diaphorase. In other words, the reaction time is short.

d) In Iwata et al. all reagents must be immobilized on the same test strip. In other words, the reagents are immobilized in different parts on the same test strip as described on page 5, lines 51 to 67. However, in the present invention, in view of storage stability, reagents are divided into three components and immobilized on two electrodes and an absorbent carrier, respectively, as specified in amended claim 8.

e) As described above, Iwata et al. discloses a general immobilization method of a test strip and spectrophotometrical measurement for formazan produced from a tetrazolium salt based on reflection light. Iwata et al. do not mention any electrochemical measurement method. In Iwata et al. all reagents must be immobilized onto the same test strip, whereas, in the present invention, the reagents are immobilized on both electrodes and absorbent carrier. This is because the method of the present invention can be attained as long as reagents are present at a reaction site on an electrode. In Example 1 of the present invention, 1-methoxy PMS instead of diaphorase is not immobilized on the absorbent carrier. 1-methoxy PMS is immobilized on an electrode in view of storage stability.

Difference between claims 1 to 3 and 8 and combination of Ishiyama, Burd and Iwata

Ishiyama et al. is concerned with a method of producing a tetrazolium salt (WST-l) for producing a water soluble formazan and colorimetric measurement. Burd et al. is concerned with an electrochemical measurement method for formazan produced from a tetrazolium salt. Iwata et al. is concerned with absorption of WST-1, dehydrogenase, NAD or NADP, and diaphorase onto a test strip. Claims 1 to 3 and 8 of the present invention seem to be analogous to the combination of Ishiyama et al. Burd et al. and Iwata et al. in respect to reagents and manufacturing method. However, they differ in at least the following points.

The formazan produced from a tetrazolium salt described by Burd et al. has been long investigated for electrochemical measurement. As a result, it is know that formazan cannot be electrochemically measured due to its irreversible reaction.

Furthermore, Burd et al. fail to describe the results of electrochemical measurement of formazan actually produced from a tetrazolium salt in Examples. The results of electrochemical measurement of fructosamine are only described. The results shown in the Examples are obtained when a potential of +1040 mV (vs. Ag/AgCl) is applied. However, at this potential, it is very difficult to practically measure a sample. It is a significant problem in constructing a biosensor.

On the other hand, WST-1 (JP 7,070,092) producing water-soluble formazan, which is not described in Burd et al, but used in the present invention, is a novel tetrazolium salt applied for a patent in 1993. Since then, whether WST-1 is electrochemically measured or not has not been investigated. Therefore, whether electrochemical measurement is impossible or not remains unproven. As a matter of fact, Ishiyama et al., who actually discovered WST-1 (Analyst, Vol. 120, p. 113 (1995) (corresponding to WST-1:1b)), *has* electrochemically measured WST-1 in accordance with a basic electrochemical measurement method, namely, cyclic voltammetry, (page 116, Fig. 5, -1000 mV to +400 mV (vs. Ag/AgCl)). However, it was concluded that no oxidation peak potential was obtained since the reaction of a tetrazolium salt is

irreversible, as mentioned above. From this paper, Ishiyama et al. clearly think that electrochemical measurement of a tetrazolium is impossible. The present inventors applied +500 mV (vs. Ag/AgCl), which Ishiyama et al. did not try, and found an intrinsic oxidation peak potential of formazan produced from WST-1 at this potential. Based on this finding, they succeeded in establishing electrochemical measurement and constructing a biosensor.

Iwata et al. discloses measurement based on reflected light but is silent about electrochemical measurement. From this, it is considered that Iwata et al. are not concerned with construction of a biosensor. However, Iwata et al. disclose a method of absorbing a water-soluble tetrazolium salt (including WST-1) and an enzyme etc., onto a test strip, which is analogous to an absorbent carrier used in the biosensor of the present invention. Since the present inventors contemplate that a reaction takes place between a working electrode and counter electrodes, applicants do not think it necessary that all reaction agents must be held on the absorbent carrier. More specifically, to improve storage stability, the reaction agents are divided into three components, which are further immobilized on both electrodes and the absorbent carrier, respectively. (WST-1 adsorbed to an absorbent carrier, a dehydrogenase, and a coenzyme are absorbed to the absorbent carrier only by virtue of the fact that they are involved in a main reaction.) However, in Iwata et al., the reaction must be carried out in a test strip, so that all reagents must be absorbed in the test strip. For the case of absorbing the reagents separately into different parts in view of storage stability, a method of impregnating different parts of a test strip with the respective reagents is described (page 5, lines 37 to 67). For example, in Iwata et al. diaphorase is also immobilized as an electron mediator, on a test strip. However, in the biosensor of the present invention, an electron mediator, 1-methoxy PMS, is immobilized on an electrode in view of storage stability.

As explained above, Burd et al. describe an electrochemical measurement of formazan produced from a tetrazolium salt. However, they fail to show experimental results. It is generally believed that formazan cannot be electrochemically measured

because of the irreversible reaction. The present inventors found an oxidation peak potential intrinsic to formazan produced from a tetrazolium salt, WST-1, which was developed by Ishiyama et al. but not described in Burd et al, in a region not measured by Ishiyama et al. Based on this finding, the present inventors established an electrochemical measurement method and constructed a biosensor. In this respect, the present invention is patentable.

Iwata et al. discloses a method of manufacturing a test strip similar to the absorbent carrier used in the biosensor of the present invention. However, Iwata et al. is neither concerned with the subjects of the present invention, more specifically, the electrochemical measurement method of formazan produced from a tetrazolium salt, nor the biosensor. Rather, Iwata et al., is concerned with a measuring method using reflected light. In the present invention, in consideration of storage stability, three types of reagents are immobilized on electrodes and an absorbent carrier respectively. However, in the method of Iwata et al, all reagents must be immobilized onto a test strip. The present invention differs from Iwata et al. in the state of immobilization.

Difference between claims 4 and 5 and Burd

Burd et al. describe a method of measuring fructosamine base on formazan produced from a tetrazolium salt by measuring a response current when a potential is applied to electrodes comprised of an anode and a cathode. However, Burd et al. differ from the present invention in the following points.

As mentioned above, it is known that formazan produced from a tetrazolium salt described in Burd et al., cannot be measured electrochemically, from the research so far performed. The present invention succeeded in establishing an electrochemical measurement method at a low potential (+500 mV (vs. Ag/AgCl)) and constructing a biosensor by using WST-1, which is not described by Burd et al.

All formazan compounds produced from tetrazolium salts disclosed by Burd et al. are hardly dissolved in water and probably cause precipitation (EP 0,908,453), which produces a big problem in developing a biosensor which enables measurement simply by

adding it to a test material to perform a reaction.

The present invention is characterized in that a biosensor is constructed by using WST-l, which is not described by Burd et al., and that a measurement method performed at a low potential is established, which is not attained by a tetrazolium salt disclosed by Burd et al. If the measurement is performed at a low potential, the effect brought by high potential application, that is, the effect of various interference substances, may be eliminated. That is, the effect of the interference substances can be minimized.

Since the formazan compounds produced from the tetrazolium salts disclosed by Burd et al. are hardly dissolved in water, precipitation will occur if the concentration of such a formazan increases. In contrast, the formazan compounds produced from WST-1 used in the present invention can be dissolved more easily than those obtained from other tetrazolium salts. Therefore, precipitation will not occur. Hence, it becomes possible to construct a biosensor capable of measuring formazan simply in a short time.

It is believed that there is a significant problem in constructing a biosensor if a tetrazolium salt disclosed by Burd et al. is used. Such a problem can be overcome by using WST-1 as a tetrazolium salt as shown in the present invention (claim 4 and 5). Based on this, the present invention established an electrochemical measurement method and constructed a biosensor. Hence applicants respectfully submit that the invention disclosed in claim 4 and 5 is fully patentable over Burd et al.

Having fully responded to all of the pending objections and rejections contained in the Official Action (Paper No. 7), applicants submit that the claims are in condition for allowance and earnestly solicit an early Notice to that effect. The examiner is invited to contact the undersigned if any further information is required.

The Terminal Disclaimer submitted March 26, 2003 (together with the appropriate recording fee) was not accepted for reasons stated on page 2 of the current Action. A new, correct Terminal Disclaimer is submitted with this response.

As the March 26, 2003 submission was not accepted, please apply the previously paid (but unused) recording fee of $110 to record the Terminal Disclaimer attached to

this response. If this is not possible, authorization is give to charge our deposit account no. 14-1140 with the relevant amount to secure recordal.

Respectfully submitted,

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